Lactational performance of Quackenbush Swiss line 5 mice

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ABSTRACT: We evaluated 2 strains of mice for their utility in the investigation of nutritional and molecular regulatory mechanisms of lactation. The lactational performance and milk composition were characterized for an inbred mouse strain, inbred Quackenbush Swiss line 5 (QSi5) selected persistently for fecundity, and a nonselected strain, CBA. The milk yield assessed by changes in BW in response to suckling of sustainable litter sizes for each strain was 3-fold greater (P < 0.001) in QSi5 mice than the CBA strain. The QSi5 mice also produced milk more efficiently (P < 0.001) than CBA mice, despite having the same quantity of mammary tissue per unit of BW. Milk composition did not vary between strains or by stage of lactation, with the exception of lactose concentration, which was greater (P = 0.003) in QSi5 mice. Expression of ε-casein was ≥10-fold greater, and αS1-casein was ≥3-fold greater, during mid and late lactation compared with early lactation in both strains, whereas κ-casein underwent an apparent alteration in posttranslational modifications in both strains from early to mid lactation. Changes in casein composition coincided with an increased susceptibility to proteolytic degradation; hence milk from early lactation may be more readily degraded to facilitate digestion in the neonate. The greater milk synthetic capacity of QSi5 mice over the lactation cycle provides a useful model for studies of nutritional and molecular regulation of lactation.

Key words: body weight, feed intake, lactation, mice, milk, protein

INTRODUCTION

Identification of mechanisms responsible for the superior lactational performance of genotypes noted for milk production may assist in the identification of rate-limiting factors for milk biosynthesis in the contemporary commercial dairy cow. The establishment of alternative models to the cow with which to explore these metabolic and molecular phenomena may have some advantages due to the long reproductive and lactational phases of the cow and the cost of animal maintenance and sampling for modern genomics and proteomics approaches. We have selected a highly fecund inbred line of mice, the inbred Quackenbush Swiss line 5 (QSi5; Holt et al., 2004) as a potential model system. The QSi5 mice have an average litter size of 13.4 (Holt et al., 2004), whereas mice of a similar genetic background, the CBA strain, have an average litter of 5.4 (Nagasawa et al., 1973). The greater reproductive capacity of QSi5 mice suggests a corresponding increase in milk biosynthetic capacity. This may lead to the identification of metabolic constraints to milk output not obvious in greater producing dams from other species, the most commercially important of which is the Holstein-Friesian cow. Changes in the pattern of milk protein synthesis during lactation may be particularly informative in promoting our understanding of these regulatory factors.

In this study, we have characterized the lactational performance of QSi5 and that of a standard mouse of similar genetic background, the CBA, over their lactation cycle. Litter size was set within the normal physiological range for each strain, namely 12 for QSi5 mice and 6 for CBA mice, to sustain lactation for both strains without compromising animal welfare. Characterization of the lactation of these mouse strains may help identify potential metabolic and molecular regulatory genes important for lactational efficiency in commercial dairy herds.
MATERIALS AND METHODS

Animals

Mice (Mus musculus) from QSi5 and CBA strains (Faculty of Veterinary Science, University of Sydney, NSW, Australia) were housed at 21°C with a daily photoperiod of 12 h (0600 to 1800). Mice had free access to water and a pelleted diet (DE content = 14.3 MJ/kg of DM, protein content = 19%; rat and mouse cubes; Specialty Feeds, Glen Forrest, WA, Australia). Seven multiparous QSi5 and 7 multiparous CBA females were mated to QSi5 and CBA males, respectively, to determine total milk production using a weigh-suckle-weigh experiment; 16 nulliparous females of each of the QSi5 and CBA strains were similarly mated for measurement of mammary gland weight, and 5 nulliparous females of each of the same strains were mated for the collection of milk samples for compositional analysis. Once pregnant, the females were caged individually. The day of parturition was deemed d 0 of lactation.

Weigh-Suckle-Weigh

Mice were offered a high-glycemic index diet (GE content = 15 MJ/kg of DM, protein content = 22%; Higgins et al., 1996) during pregnancy and lactation to ensure that milk production was not limited by energy or protein intake. This consisted of (as-fed basis) 514 g of glucose/kg (Glucodin, Boots Healthcare, North Ryde, NSW, Australia), 85 g of sucrose/kg, 50 g of wheat bran/kg, 200 g of casein/kg (P. T. Cheil Samsung, Jawa Timur, Indonesia), 50 mL of canola oil/kg, 67 g of mineral mixture/kg (ICN Biochemicals, Aurora, OH), 13 g of vitamin mixture/kg (Uni Rat Vitamin, International Animal Health Products, Huntingwood, NSW, Australia), 19 g of gelatin/kg, and 2 g of DL-methionine/kg (Nippon Soda Company, Chiyoda-ku Tokyo, Japan).

At parturition, QSi5 pups were decreased to 12 and CBA litters were adjusted to 6 by cross-fostering of pups to maintain normal physiological states for both strains. Milk yield and pup growth were determined from d 1 to 18 of lactation using the weigh-suckle-weigh method described by Sampson and Jansen (1984), with the exception that whole litters were weighed in the current study rather than individual pups. In brief, litters were separated from their dams to a thermoneutral environment for 4 h and returned to suckle for 2 h. This 6-h cycle continued for 24 h and was performed for 18 d of the cycle. Corrections were made for BW loss associated with metabolic processes by monitoring of BW changes over the 4-h separation periods and extrapolating this to 24 h (Reddy and Donker, 1965). Dams were weighed daily, and daily feed intakes were recorded. Mean BW was used to predict basal metabolic rate (BMR) for each strain, by the application of Kleiber’s law (Smil, 2000).

Mammary Gland Weights

Mammary glands were removed from QSi5 dams that had suckled 9 to 12 pups and CBA dams that had suckled 3 to 6 pups. Mice were euthanized with CO2 on d 0, 8, 15, and 22 of lactation. The BW was recorded and the fourth (inguinal) mammary gland from the left and right side of each animal was removed. Each gland was weighed separately.

Mouse Milking

For compositional analysis, the QSi5 mice suckling 10 to 14 pups and CBA mice suckling 4 to 6 pups were milked at early (d 2 to 3), mid (d 9 to 10) and late (d 18 to 19) lactation. Pups were removed 2 h before milking and placed near a lamp to maintain thermoneutrality during separation. Dams were weighed, and 25 g of 2,2,2 tribromoethanol/L (Aldrich Chemical Co., Milwaukee, WI) in 25 mL of tert-amyl alcohol/L was administered intraperitoneally at a rate of 0.01 mL/g of BW to anesthetize the animal. Dams were then intraperitoneally injected with 0.5 IU of synthetic oxytocin (Troy Laboratories Pty., Ltd., Smithfield, NSW, Australia). After 5 min, a tube (1.0 mm i.d.) was placed over each teat and a light vacuum applied. Each mouse was milked for approximately 10 min. Milk was collected into a sterile microcentrifuge tube and then stored in aliquots at −80°C. Mice were placed on a warming pad until fully recovered and then returned to their litter.

Analysis of Mouse Milk

Protein concentration of mouse milk was determined using a colorimetric assay (2D-Quant kit, Amersham Biosciences SF Corp., San Francisco, CA). Milk was deproteinized by trichloroacetic acid precipitation and then neutralized with KOH before analysis for lactose and urea. Lactose and urea concentrations were determined using enzymatic analysis kits for lactose/D-glucose and urea/ammonia (R-Biopharm GmbH, Darmstadt, Germany). The lactose/D-glucose kit is based on methods described by Beutler (1984) and Kunst et al. (1984) and the principles of the urea/ammonia kit from Kerscher and Ziegenhorn (1985). Fat content was determined as described by Knight et al. (1986) using microhematocrit tubes (75-mm long, 0.5 to 0.6 mm i.d.; Brand GmbH, Wertheim, Germany).

Gel Electrophoresis

Equivalent amounts of protein from milk collected on d 2, 10, and 18 of lactation from each strain were analyzed by 12% SDS-PAGE (Laemmli, 1970). Milk from 3 QSi5 mice and 2 CBA mice was also analyzed by 2-dimensional gel electrophoresis (2-DE). Each sample was run at least twice to confirm reproducibility of results. Mouse milk protein (200 μg) was rehydrated into a 13-cm, pH 4 to 7, immobilized pH-gradient (IPG) strip (Amersham Biosciences, Uppsala, Sweden) in 8 M urea,
20 g of 3-[(3-cholamidopropyl)dimethylammonio]-1-propane sulfonate/L, 20 mL of IPG buffer/L, pH 4 to 7 (Amersham Biosciences), and 18 mM dithiothreitol with and without mammalian protease inhibitor cocktail (Sigma-Aldrich Co., St. Louis, MO).

The IPG strips were run on a Multiphor II apparatus (Amersham Biosciences) for 21 kV-h consisting of a 0- to 300-V gradient for 1 min, then a 300- to 3,500-V gradient over 90 min, followed by 4 h at 3500 V. The IPG strips were then equilibrated for 15 min in 0.05 M Tris, pH 8.8, 6 M urea, 300 mL of glycerol/L, 20 g of SDS/L, 0.02 g of bromophenol blue/L, 10 g of DTT/L, followed by 15 min in 0.05 M Tris, pH 8.8, 6 M urea, 300 mL of glycerol/L, 20 g of SDS/L, 0.02 g of bromophenol blue/L, and 25 g of iodoacetamide/L. Strips were then run on 15% SDS-PAGE gels (20 cm × 1 mm). Gels were stained with freshly prepared colloidal Coomassie blue (1 g of Coomassie blue G-250/L, 170 g of ammonium sulfate/L, 340 mL of methanol/L, and 30 mL of o-phosphoric acid/L).

Gel images were analyzed using ImageMaster 2D Platinum v5.0 (Amersham Biosciences). For each mouse strain, protein spots were matched between gels of milk from d 2, 10, and 18 of lactation. Protein spots were also matched between strains for d 2, 10, and 18 of lactation. Spot volumes were calculated, and spot volume ratios between days of lactation or between strains for d 2, 10, and 18 were also matched between strains for d 2, 10, and 18 of lactation. Protein spots were stained with freshly prepared colloidal Coomassie blue (1 g of Coomassie blue G-250/L, 170 g of ammonium sulfate/L, 340 mL of methanol/L, and 30 mL of o-phosphoric acid/L).

**Mass Spectrometry**

In-gel tryptic digests were performed on selected gel spots for identification of proteins. Protein spots were digested overnight using 200 ng of trypsin (EC No. 232 650 8; Sigma-Aldrich) in 0.05 M ammonium bicarbonate. De novo peptide sequencing by electrospray ionization tandem mass spectrometry (ESI-MS/MS) was undertaken at the Biomedical Mass Spectrometry Facility, University of New South Wales, Sydney, Australia. Proteins were identified from mass spectrometry results using the Mascot search engine (Perkins et al., 1999).

**Statistical Analyses**

Repeated measures ANOVA (GenStat 7.0, Lawes Agricultural Trust, Rothamsted Experimental Station, UK) was used to assess differences in efficiency of feed use, milk yield per pup, milk yield per gram of dam BW, and milk composition of QSi5 and CBA mice. The statistical models fitted to the data were of the form

\[ Y_{ijk} = \mu + \text{Strain}_i + \text{Day}_j + (\text{Strain} \times \text{Day})_{ij} + (\text{Strain} \times \text{Mouse})_{ik} + \varepsilon_{ijk}, \]

where \( Y_{ijk} \) is the efficiency of feed use, milk yield per pup, milk yield per gram dam BW, or composition measure; \( \mu \) is the overall mean for that measure; \( \text{Strain}_i \) is the effect of CBA vs. QSi5; \( \text{Day}_j \) assesses the effect of the day of lactation; \( (\text{Strain} \times \text{Day})_{ij} \) is the interaction of strain and day; \( (\text{Strain} \times \text{Mouse})_{ik} \) is a random effect associated with the lactation record for mouse \( k \) within strain \( I \); and \( \varepsilon_{ijk} \) is the residual random error. Greenhouse-Geisser adjustments were included to allow for the serial correlation of the random errors. Strain comparisons at particular times were assessed by least significant differences. A “broken stick” regression model was fitted to the milk yield (MY) data to assess a change around d 6. The statistical model was of the form

\[ \text{MY}_{ijk} = \mu + \text{Strain}_i + \beta_1 \text{Days}_j + \beta_2 \text{Days}_6j + \beta_3 (\text{Strain} \times \text{Days})_{ij} + \beta_4 (\text{Strain} \times \text{Days}_6)_{ij} + \text{Strain} \times \text{Mouse}_{ik} + \varepsilon_{ijk}, \]

where Days6 = 0 if Days < 6, or Days6 = Days − 6 if Days ≥ 6.

Strain-specific linear trends in dam BW were assessed by fitting GLM with separate slopes for each strain. Strain differences in total milk yield, BMR, and BMR/g of BW were assessed using a 2 sample t-test. The relative mammary gland weight (average mammary gland weight as a percentage of total BW) was evaluated at d 0, 8, 15, and 22 of lactation for both strains of mice. Strain and day differences were evaluated using GLM for unbalanced ANOVA.

**RESULTS**

**Milk Yield and Efficiency of Production**

Milk output from QSi5 mice rose rapidly through to d 6 of lactation after which the increase was more modest (change in regression slopes: \( P < 0.001 \)) to attain a value of 9.8 g of milk per day by d 18. Milk output was almost linear for the duration of lactation in the CBA mice with a maximum production of 4.0 g/d (Figure 1a). Because of pup deaths during the course of the experiment, the average number of pups over the lactation was 11.9 for QSi5 mice and 5.0 for CBA mice. The QSi5 mice produced more milk per pup up to d 10 of lactation (\( P < 0.05 \) on d 2 to 6 and 9), whereas in late lactation both strains produced similar yields per pup (Figure 1b). Over the entire lactation QSi5 mice produced a total of 119.9 ± 3.8 g of milk, an average of 10.1 g of milk/pup. The CBA mice produced 40.3 ± 5.8 g of milk over the entire lactation, an average of 8.3 g of milk/pup.

Lactating QSi5 mice had an average BW of 40.9 ± 0.2 g, whereas CBA dams averaged 25.5 ± 0.4 g. There was no significant change in QSi5 dam BW during lactation, whereas in CBA mice there was a small increase (\( P < 0.001 \)) in BW (0.26 g/d; Figure 1c). Lactating QSi5 mice had a greater predicted BMR (0.31 W) than CBA dams (0.21 W; \( P < 0.001 \)). However, when adjusted for differences in BW, CBA mice displayed a BMR per gram of BW 1.13 times greater than QSi5 dams (\( P < 0.001 \)). Over the entire lactation, QSi5 mice produced on aver-
Lactational performance of mice

Figure 1. Milk yields from inbred Quackenbush Swiss line 5 (QSi5; ○) and CBA (●) mice were determined for each day of lactation using the weigh-suckle-weigh technique. (A) milk yield per day, (B) milk yield per pup, (C) dam BW, and (D) milk yield per gram of feed consumed. Error bars represent the LSD between strain means at any given day of lactation.

age 0.163 ± 0.004 g of milk per gram of dam BW, and CBA mice produced 0.084 ± 0.011 g of milk per gram of dam BW (P < 0.001).

The QSi5 mice consumed between 8.1 ± 0.7 and 19.1 ± 1.1 g of feed per day (272.7 ± 23.4 g over the entire lactation), whereas CBA dams consumed between 4.1 ± 0.5 and 12.6 ± 1.3 g of feed per day (158.0 ± 14.9 g). There was no interaction of day × strain; however, the QSi5 dams produced more (P < 0.001) milk per gram of feed than their CBA counterparts throughout lactation (Figure 1d).

Mammary Gland Weights

There was no difference in the mean relative mammary gland weight between QSi5 and CBA dams over the lactation (QSi5 1.16%, CBA 1.06%, P = 0.35), nor was there any interaction between day and strain (P = 0.42).

Mouse Milk Composition

Milk was successfully collected from 3 QSi5 females and 3 CBA females on d 2 or 3, d 9 or 10, and d 17 or 18 of lactation representing early, mid, and late lactation respectively. On average, 140 µL of milk was obtained per milking from QSi5 mice and 70 µL of milk was obtained per milking from CBA mice. Protein, fat, lactose, and urea concentrations of mouse milk were determined where sufficient milk was available (Table 1). There was no interaction of day and strain for the various milk components. Lactose concentration was greater (P = 0.003) in QSi5 mice compared with CBA mice. There was no other difference in milk composition between strains, nor did the composition of QSi5 milk vary significantly throughout lactation.

Analysis of Mouse Milk Protein

The 5 caseins in mouse milk were successfully identified from 12% SDS-PAGE by ESI-MS/MS (Figure 2). All the caseins ran at molecular weights above those predicted from their amino acid sequence (αS1-casein 34 kDa, β-casein 24 kDa, κ-casein 18 kDa, γ-casein 19 kDa, and ε-casein 15 kDa). No visual difference was observed between the protein profiles of milk from QSi5 and CBA mice; however, changes were observed in both strains depending on the stage of lactation. Given that equivalent amounts of total protein were loaded in each lane, it appeared that ε-casein was expressed at lower
Table 1. Composition of milk from inbred Quackenbush Swiss line 5 (QSi5) and CBA mice

<table>
<thead>
<tr>
<th>Item</th>
<th>Lactose, g/L</th>
<th>Protein, g/L</th>
<th>Urea, g/L</th>
<th>Fat, %</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mouse strain</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>QSi5</td>
<td>21.2± 1.3 (n = 9)</td>
<td>87.6 ± 7.7 (n = 8)</td>
<td>0.44 ± 0.03 (n = 9)</td>
<td>28.3 ± 3.2 (n = 3)</td>
</tr>
<tr>
<td>CBA</td>
<td>17.7± 1.2 (n = 3)</td>
<td>91.6 ± 8.9 (n = 5)</td>
<td>0.36 ± 0.05 (n = 3)</td>
<td>28.4 ± 1.0 (n = 3)</td>
</tr>
<tr>
<td><strong>Day of lactation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 to 3</td>
<td>17.3 ± 0.5 (n = 3)</td>
<td>67.2 ± 10.6 (n = 3)</td>
<td>0.40 ± 0.05 (n = 3)</td>
<td>—</td>
</tr>
<tr>
<td>10</td>
<td>20.9 ± 1.2 (n = 3)</td>
<td>103.9 ± 3.9 (n = 3)</td>
<td>0.52 ± 0.03 (n = 3)</td>
<td>—</td>
</tr>
<tr>
<td>17 to 18</td>
<td>25.3 ± 1.4 (n = 3)</td>
<td>91.7 ± 21.3 (n = 2)</td>
<td>0.39 ± 0.08 (n = 3)</td>
<td>—</td>
</tr>
</tbody>
</table>

1The top half of the table shows the comparison of milk composition between strains, averaged over the lactation, and the bottom half of the table shows the composition of QSi5 milk at different stages of lactation. **Means ± SEM differ (P < 0.001).**

Several proteins from these gels were identified by ESI-MS/MS analysis (Figure 3). In most cases, individual proteins were represented by more than 1 spot. Mature forms of αS1-casein (spot 1a), β-casein (spot 3), γ-casein (spot 4a), ε-casein (spot 5a), and whey acidic protein (spot 6a) were identified by ESI-MS/MS. The lower molecular weight forms were inconsistent with the molecular weight of the intact mature proteins. Given the reduction in the intensity of the lower molecular weight spots (spots 1b, 4b, 5b, 6b) in the presence of protease inhibitors, we conclude that they represented proteolytic fragments of mature proteins. An intact form of κ-casein was not identified from the 2-DE. Based on its molecular weight, spot 2 was assessed to be a proteolytic fragment of κ-casein.

In addition to changes in the protein profiles due to proteolysis, some changes in milk protein expression levels on 2-DE were also detected using image analysis software. In the presence of protease inhibitors, expression of ε-casein was consistently ≥10-fold greater on d 10 and 18 compared with d 2 (Figure 3b). The αS1-casein expression level increased 5-fold from d 2 to 10 and increased 3-fold on d 18 compared with d 2, although this was not observed for all samples. In some cases, the change in expression was ≤2-fold due to greater expression levels on d 2, whereas in a sample collected on d 3 instead of d 2 the level of αS1-casein expression was equivalent to those typically observed on d 10 of lactation (data not shown). No change in the level of expression of β-casein, γ-casein, or whey acidic protein was detected during lactation. No differential protein expression was detected between strains.

DISCUSSION

The lactational performance of QSi5 and CBA mice were evaluated as models for studying various aspects of lactation. Litter sizes used in the study were based on previous experience and published data on these 2 mouse strains (Nagasawa et al., 1973; Holt et al., 2004). Thus, QSi5 and CBA mice raised litters of average size...
Figure 3. Two-dimensional gel electrophoresis of inbred Quackenbush Swiss line 5 (QSi5) mouse milk from d 2, 10, and 18 of lactation in the absence (A) and presence (B) of protease inhibitors. The spots indicated with circles or ovals were identified by electrospray ionization tandem mass spectrometry as: αS1-casein (1a), αS1-casein fragment (1b), κ-casein fragment (2), β-casein (3), γ-casein (4a), γ-casein fragment (4b), ε-casein (5a & 5b), whey acidic protein (6a), whey acidic protein fragment (6b), and serum albumin fragments (7a & 7b).
of BW. Given that dams from both strains maintained their BW throughout lactation, QSi5 mice directed more dietary nutrients to milk than the CBA dams, producing up to 4 times more milk per gram of feed consumed. Comparative genomic studies have yielded complex expression patterns of genes responsible for the use of energy substrates in tissues, many of which relate to their sensitivity to the actions of the key metabolic regulator insulin (Yechoor et al., 2002). Insulin also plays an integral role in the endocrine lactogenic complex (Neville et al., 2002). This sensitivity may contribute to the innate lactational efficiency of the mammary epithelium in the QSi5 genotype. The relative ease with which the energy status of monogastric species can be manipulated by dietary means, it is likely that this strain can be used to identify nutritional and endocrine limitations to the partitioning of energy substrate to the mammary gland for milk biosynthesis.

The separation of pups and dams required for the weigh-suckle-weigh technique suppresses the growth rate of pups by up to 38% (Sampson and Jansen, 1984); therefore this method may underestimate lactational potential. However, in a subsequent study conducted on QSi5 mice in which pups were not removed from their dams, pups had similar growth rates to the current study (data not shown). We suggest that, over the lactation cycle, suckling pups were able to compensate for the periods of separation required by the weigh-suckle-weigh procedure. Alternative methods for measuring milk yield, such as the isotope methods employed by Rath and Thenen (1979) and Knight et al. (1986), typically give rise to greater estimates of milk production compared with the weigh-suckle-weigh method. Whereas milk production levels comparable or greater than those for QSi5 mice have been reported for outbred strains (Jara-Almonte and White, 1972; Rath and Thenen, 1979; Knight et al., 1986), we believe that this is the first report of a highly productive inbred strain. This strain therefore provides a useful model for genomic and proteomic approaches to understanding lactational regulation at the molecular level.

The increased lactational output of QSi5 mice did not alter milk composition. Protein, lactose, and fat concentrations in QSi5 milk were similar to those reported in other strains (Ragueneau, 1987; Croke-Auld, 2002). Lactose concentration was significantly greater in milk from QSi5 mice than in the CBA strain. Although increased lactose concentration is usually associated with more dilute milk (Davis, 1997), protein and fat concentrations in QSi5 milk were maintained at levels similar to those in CBA mice. The urea concentrations were comparable with those in nonlactating mice reported by Al Banchaabouchi et al. (2001). Urea concentrations were also indicative of metabolic efficiency of dietary protein use and indirectly of energy status because caloric restriction increases urea output (Tillman et al., 1996). Urea concentrations did not significantly change throughout lactation, which was consistent with the maintenance of dam BW throughout lactation by increasing feed intake. We conclude that the efficiency of dietary protein use did not vary over the lactation cycle.

The 5 caseins reported in mouse milk (Hennighausen and Sippel, 1982; Rijnkels et al., 1997) were identified on SDS-PAGE and 2-DE. All of the caseins ran at positions above their predicted molecular weights, as reported elsewhere (Green and Pastewka, 1976). Expression of ε-casein increased 10-fold from early lactation to mid lactation. Consistent with this, Rijnkels et al. (1997) observed low levels of ε-casein mRNA (also known as δ-casein) in mouse mammary gland during early lactation compared with peak lactation. Yoneda et al. (2001) also reported that an apparent 23-kDa protein, now known to be ε-casein, was seen in mature mouse milk (d 8) but not in colostrum (d 0). This group also found that αS1-casein levels were lower in colostrum than in mature milk. In the current study, αS1-casein expression began to increase between d 2 and 3 of lactation. κ-Casein appeared to undergo a change in the level of posttranslational modification throughout lactation. During early lactation, κ-casein gave rise to an indistinct band on SDS-PAGE, which was indicative of a highly glycosylated protein (Green and Pastewka, 1976). This form decreased, and a distinct band of lower apparent molecular weight appeared as lactation progressed, indicative of a less glycosylated form of κ-casein. This protein was only identified as a proteolytic fragment on 2-DE. It is possible that κ-casein is more susceptible to proteolytic degradation than other caseins. It is known that bovine κ-casein is highly susceptible to proteolytic degradation by rennin (Green and Pastewka, 1976). The αS1-casein, β-casein, γ-casein, and ε-casein spots identified on 2-DE each sat within a horizontal group of spots, which most likely represented the same protein with different posttranslational modifications (Liebler, 2002). Casein expression levels remain relatively constant throughout lactation in dairy cattle (Walker et al., 2004); therefore longitudinal studies of factors regulating changes in casein expression in mice may identify genes regulating casein expression, which in turn may be useful for elucidating similar mechanisms in the dairy cow.

In the absence of protease inhibitors, mouse milk proteins were more susceptible to proteolytic degradation under 2-DE conditions. This probably occurred during overnight rehydration of the IPG strips because no evidence of sample degradation was observed on SDS-PAGE. Milk from early lactation was more susceptible to degradation than milk from mid and late lactation. Interestingly, the changes in casein expression appeared to coincide with a change in susceptibility to proteolytic degradation of murine milk. The changes in expression levels of ε-casein and αS1-casein, and the alterations in posttranslational modifications of κ-casein, may alter micelle composition and stability, rendering them more susceptible to degradation during early lactation. This may facilitate digestion of milk protein by neonatal mice, which is important for in-
creasing nutrient availability for growth and for the release of key developmental peptides. Yoneda et al. (2001) found that caseins in colostrum were more readily digested by neonatal mice than casein from mature milk. The reason for this differential susceptibility to degradation may be as much due to the characteristics of the milk proteins as to changes in the gastrointestinal milieu with age. The peptides emanating from this digestion may act as a source of novel bioactive agents.

The QSi5 mice strain will be useful in exploring various aspects of lactation. Further investigation of milk protein peptides from early lactation may provide a novel source of bioactive agents. We conclude that the changes in casein expression and the greater milk biosynthetic capacity of the QSi5 mouse make it a suitable model for the identification of molecular and regulatory mechanisms that influence lactational output.

LITERATURE CITED


